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Behaviour of selected anti-retrovirals' active pharmaceutical ingredients and the assessment of their bacterial viability in a simulated wastewater treatment plant

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ABSTRACT

The release of micropollutants into the environment via wastewater treatment plants (WWTP) is one of the issues which has long been considered a major source of environmental contamination due to the ineffectiveness of WWTPs in eliminating them. In these releases, a wide range of compounds is measured in trace amounts (ng/L to µg/L) such as pharmaceutical residues and hormones (PPHs), pesticides, phthalates, artificial sweeteners, chemical products, and personal care products. This study examined the effects of nevirapine, a non-nucleoside reverse transcriptase inhibitor (NNRTI) drug and with lamivudine, a nucleoside reverse transcriptase inhibitor (NRTI) drug in combination, on a simulated activated sludge process using actual wastewater from Bushkoppie Wastewater Treatment in Johannesburg, South Africa. Laboratory experiments were performed using duplicate samples of raw influent collected during June 2018 while the final laboratory experiments were performed using triplicate samples collected from raw influent during November 2018. 1, 15, and 25 mg/L concentrations of nevirapine were introduced into the wastewater and the concentrations of chemical oxygen demand (COD) and ammonia-nitrogen in the effluent of each experiment are recorded on a 2-hly basis until the 8th h when the experiment was terminated. Samples with 15 mg/L nevirapine inhibited the specific concentration variations of COD by 52.9% (standard deviation 27%) and the specific $N\text{-}NH_4^+$ concentration variation by 30% (standard deviation 21%). An increase in COD, as well as a decrease in total suspended solids (TSS), were observed in the wastewater with nevirapine. In order to assess nevirapine's time-kill activity, continuous experiments were conducted both in closed mode (batch equivalent) and imaging techniques combined with an L7007 LIVE/DEAD BacLight viability kit (Invitrogen, South Africa). The nevirapine toxicity in the wastewater was observed at lower concentration when exposure time increased. A 0.1 mg/L nevirapine concentration was toxic to heterotrophic bacteria on a closed mode, and inhibited nitrification. These findings agree with the microscopic studies, which showed a latency time before the lower nevirapine concentrations began to kill the bacteria. After 40 minutes there were 97 % (Standard Deviation 3.8) of living bacteria in control reactors, 76 % (Standard Deviation 3.1) in

reactors that contained 0.1 mg/L nevirapine and 46 % (Standard Deviation 18.6) in the system that contained 10 mg/L nevirapine.

The influence of nevirapine and lamivudine on municipal sludge in batch reactors focuses on extracellular polymeric substances (EPS) as an indicator of bacteria sensitivity with respect to the above-mentioned drugs. The EPS were analyzed by FT-IR spectroscopies. It was found that both drugs induced a significant increase of bound EPS in flocs. This may be attributed to a protection mechanism by the bacteria. However, only Nevirapine inhibited COD and nitrogen removal.

Key words: WWTP, Nevirapine, COD, Lamivudine, nitrification, sludge extracellular polymers substances, inhibition, emerging contaminants, HIV, ammonia-nitrogen